

CLAIMS

What is claimed is:

- 1 ~~X~~. An isolated nucleic acid encoding at least a functional fragment of a 3-OST
2 protein.
- 1 2. An isolated nucleic acid as in claim 1 wherein said nucleic acid encodes a 3-
2 OST protein comprising a mature 3-OST-1 protein selected from the group consisting
3 of mature murine 3-OST-1 and mature human 3-OST-1.
- 1 3. An isolated nucleic acid as in claim 1 wherein said nucleic acid encodes a 3-
2 OST protein comprising a protein selected from the group consisting of 3-OST-1, 3-
3 OST-2, 3-OST-3A, 3-OST-3B, 3-OST-4, and ce3-OST.
- 1 4. An isolated nucleic acid as in claim 1 wherein said nucleic acid encodes a 3-O-
2 sulfotransferase domain of a 3-OST protein selected from the group consisting of 3-
3 OST-1, 3-OST-2, 3-OST-3A, 3-OST-3B, 3-OST-4, ce3-OST.
- 1 5. An isolated nucleic acid as in claim 1 wherein said nucleic acid comprises a
2 nucleotide sequence selected from nucleotide sequences within:
3 (a) SEQ ID NO: 1;
4 (b) SEQ ID NO: 3;
5 (c) SEQ ID NO: 5;
6 (d) SEQ ID NO: 7;
7 (e) SEQ ID NO: 9;
8 (f) SEQ ID NO: 11;
9 (g) a sequence having at least 60% nucleotide sequence identity with at least
10 one of (a)-(f) and encoding a functional fragment having sequence-specific HS
11 binding affinity or 3-O-sulfotransferase activity; and
12 (h) a sequence differing from a sequence of (a)-(g) only by the substitution of
13 synonymous codons.
- 1 6. An isolated nucleic acid as in claim 1 wherein said nucleic acid comprises a
2 nucleotide sequence encoding a polypeptide selected from the group consisting of:
3 (a) residues 21-52, 260-269, 250-276, 53-311, or 21-307 of SEQ ID NO: 2;
4 (b) residues 21-48, 256-265, 246-272, 49-307, or 21-303 of SEQ ID NO: 4;
5 (c) residues 42-109, 313-325, 303-332, or 110-367 of SEQ ID NO: 6;
6 (d) residues 44-147, 351-363, 341-370, or 148-406 of SEQ ID NO: 8;

- 7 (e) residues 66-132, 336-348, 326-355, or 133-390 of SEQ ID NO: 10;
8 (f) residues 396-408, 386-4150, or 207-456 of SEQ ID NO: 12;
9 (g) residues 240-250, 230-257, 23-291 of SEQ ID NO: 15;
10 (h) a sequence having at least 60% amino acid sequence similarity with at
11 least one of (a)-(g) and encoding a functional fragment having sequence-specific HS
12 binding affinity or 3-O-sulfotransferase activity; and
13 (i) a sequence comprising a chimera of at least two of sequences (a)-(h).

1 7. An isolated nucleic acid comprising at least 16 consecutive nucleotides of a
2 nucleotide sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID
3 NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, and SEQ ID NO: 11.

1 8. A host cell transformed with a nucleic acid of any one of claims 1-7, or a
2 descendant thereof.

1 9. A host cell as in claim 8 wherein said host cell is selected from the group
2 consisting of bacterial cells, yeast cells, and insect cells.

1 10. A host cell as in claim 8 wherein said host cell is selected from the group
2 consisting of somatic cells, fetal cells, embryonic stem cells, zygotes, gametes, germ
3 line cells, and transgenic animal cells.

1 11. A host cell as in claim 8 wherein said cell is a mammalian cell.

1 12. A host cell as in claim 11 wherein said cell is selected from the group
2 consisting of COS-7 cells, CHO, murine primary cardiac microvascular endothelial
3 cells (CME), murine mast cell line C57.1, human primary endothelial cells of
4 umbilical vein (HUVEC), F9 embryonal carcinoma cells, rat fat pad endothelial cells
5 (RFPEC), L cells, and cells derived from the transgenic animals of the invention.

1 13. A substantially pure protein preparation comprising at least a functional
2 fragment of a 3-OST protein.

1 14. A substantially pure protein preparation as in claim 13 wherein said 3-OST
2 protein is selected from the group consisting of mature murine 3-OST-1 and mature
3 human 3-OST-1.

1 15. A substantially pure protein as in claim 13 wherein said 3-OST protein is
2 selected from the group consisting of 3-OST-1, 3-OST-2, 3-OST-3A, 3-OST-3B, 3-
3 OST-4, and ce3-OST.

1 16. A substantially pure protein preparation as in claim 13 wherein said functional
2 fragment comprises a 3-O-sulfotransferase domain of a 3-OST protein selected from
3 the group consisting of 3-OST-1, 3-OST-2, 3-OST-3A, 3-OST-3B, 3-OST-4, and ce3-
4 OST.

1 17. A substantially pure protein preparation as in claim 13 wherein said functional
2 fragment comprises an amino acid sequence selected from amino acid sequences
3 within:

- 4 (a) SEQ ID NO: 2;
- 5 (b) SEQ ID NO: 4;
- 6 (c) SEQ ID NO: 6;
- 7 (d) SEQ ID NO: 8;
- 8 (e) SEQ ID NO: 10;
- 9 (f) SEQ ID NO: 12;
- 10 (g) SEQ ID NO: 15;
- 11 (h) a sequence having at least 60% amino acid similarity with at least one of
- 12 (a)-(g) and having sequence-specific HS binding affinity or 3-O-sulfotransferase
- 13 activity; and
- 14 (i) a sequence comprising a chimera of at least two of sequences (a)-(h).

1 18. A substantially pure protein preparation as in claim 13 wherein said functional
2 fragment comprises an amino acid sequence selected from the group consisting of:

- 3 (a) residues 21-52, 260-269, 250-276, 53-311, or 21-307 of SEQ ID NO: 2;
- 4 (b) residues 21-48, 256-265, 246-272, 49-307, or 21-303 of SEQ ID NO: 4;
- 5 (c) residues 42-109, 313-325, 303-332, or 110-367 of SEQ ID NO: 6;
- 6 (d) residues 44-147, 351-363, 341-370, or 148-406 of SEQ ID NO: 8;
- 7 (e) residues 66-132, 336-348, 326-355, or 133-390 of SEQ ID NO: 10;
- 8 (f) residues 396-408, 386-415, or 207-456 of SEQ ID NO: 12;
- 9 (g) residues 240-250, 230-257, 23-291 of SEQ ID NO: 15;

10 (h) a sequence having at least 60% amino acid sequence similarity with at
11 least one of (a)-(g) and encoding a functional fragment having sequence-specific HS
12 binding affinity or 3-O-sulfotransferase activity; and

13 (i) a sequence comprising a chimera of at least two of sequences (a)-(h).

1 ~~19.~~ A method of 3-O-sulfating saccharide residues within a preparation of
2 glycosaminoglycan or proteoglycan polysaccharides comprising:
3 contacting said preparation with at least a 3-O-sulfotransferase domain of a 3-
4 OST protein in the presence of a sulfate donor under conditions which permit
5 sulfation of said residues;

6 wherein, said 3-OST protein is selected from the group consisting of 3-OST-1,
7 3-OST-2, 3-OST-3A, 3-OST-3B, 3-OST-4, ce3-OST, and conservative substitution
8 variants or chimeras thereof.

1 ~~20.~~ A method of 3-O-sulfating saccharide residues within a preparation of
2 glycosaminoglycan or proteoglycan polysaccharides, wherein said polysaccharides
3 include a polysaccharide sequence of GlcA→GlcNS ±6S comprising:
4 contacting said preparation with a 3-OST-1 protein in the presence of a sulfate
5 donor under conditions which permit said 3-OST-1 to convert said GlcA→GlcNS ±6S
6 sequence to GlcA→GlcNS 3S ±6S.

7 wherein the 3-OST-1 protein is selected from the group consisting of murine
8 3-OST-1, human 3-OST-1, mature murine 3-OST-1, mature human 3-OST-1, a
9 functional fragment of a 3-OST-1 having 3-O-sulfotransferase activity, a conservative
10 substitution variant of 3-OST-1 having 3-O-sulfotransferase activity, and a chimeric
11 3-OST-1 having 3-O-sulfotransferase activity.

1 21. A method as in claim 20, wherein said GlcA→GlcNS ±6S polysaccharide
2 sequence comprises a part of a polysaccharide sequence selected from the group
3 consisting of:

- 4 (a) GlcA→GlcNS ±6S→IdoA 2S→ GlcNS ±6S;
5 (b) IdoA→GlcNAc 6S→GlcA→GlcNS ±6S→IdoA 2S→ GlcNS 6S;
6 (c) IdoA→GlcNS 6S→GlcA→GlcNS ±6S→IdoA 2S→ GlcNS 6S;
7 (d) IdoA→GlcNAc→GlcA→GlcNS ±6S→IdoA 2S→ GlcNS 6S;
8 (e) IdoA→GlcNS→GlcA→GlcNS ±6S→IdoA 2S→ GlcNS 6S;

9 (f) IdoA→GlcNAc 6S→GlcA→GlcNS ±6S→IdoA 2S→GlcNS;

10 (g) IdoA→GlcNS 6S→GlcA→GlcNS ±6S→IdoA 2S→GlcNS;

1 ~~22.~~ A method of 3-O-sulfating saccharide residues within a preparation of
2 glycosaminoglycan or proteoglycan polysaccharides, wherein said polysaccharides
3 include a polysaccharide sequence of GlcA 2S→GlcNS comprising:
4 contacting said preparation with a 3-OST-2 protein in the presence of a sulfate
5 donor under conditions which permit said 3-OST-2 to convert said GlcA 2S→GlcNS
6 sequence to GlcA 2S→GlcNS 3S.

7 wherein the 3-OST-2 protein is selected from the group consisting of 3-OST-2,
8 a functional fragment of a 3-OST-2 having 3-O-sulfotransferase activity, a
9 conservative substitution variant of 3-OST-2 having 3-O-sulfotransferase activity, and
10 a chimeric 3-OST-2 having 3-O-sulfotransferase activity.

1 23. A method as in claim 22, wherein said GlcA 2S→GlcNS polysaccharide
2 sequence comprises a part of a GlcNS→GlcA 2S→GlcNS polysaccharide sequence.

1 ~~24.~~ A method of 3-O-sulfating saccharide residues within a preparation of
2 glycosaminoglycan or proteoglycan polysaccharides, wherein said polysaccharides
3 include a polysaccharide sequence of IdoA 2S→GlcNS comprising:
4 contacting said preparation with a 3-OST-3 protein in the presence of a sulfate
5 donor under conditions which permit said 3-OST-3 to convert said IdoA 2S→GlcNS
6 sequence to IdoA 2S→GlcNS 3S.

7 wherein the 3-OST-3 protein is selected from the group consisting of 3-OST-
8 3A, 3-OST-3B, a functional fragment of a 3-OST-3 having 3-O-sulfotransferase
9 activity, a conservative substitution variant of 3-OST-3 having 3-O-sulfotransferase
10 activity, and a chimeric 3-OST-3 having 3-O-sulfotransferase activity.

1 25. A method as in claim 24, wherein said IdoA 2S→GlcNS polysaccharide
2 sequence comprises a part of a GlcNS→IdoA 2S→GlcNS polysaccharide sequence.

1 ~~26.~~ A method for enriching the AT-binding fraction in a preparation of heparan
2 sulfates, wherein said preparation includes a polysaccharide sequence of
3 GlcA→GlcNS ±6S comprising:

4 contacting said preparation with 3-OST-1 protein in the presence of a sulfate
5 donor under conditions which permit said 3-OST-1 to convert said GlcA→GlcNS ±6S
6 sequence to GlcA→GlcNS 3S ±6S, thereby increasing the fraction of AT-binding
7 heparan sulfates.

1 ~~27.~~ A method for converting HS^{act} precursor to HS^{act} in a preparation of heparan
2 sulfates, wherein said preparation includes HS^{act} precursor polysaccharides including
3 a sequence of GlcA→GlcNS ±6S comprising:

4 contacting said preparation with 3-OST-1 protein in the presence of a sulfate
5 donor under conditions which permit said 3-OST-1 to convert said GlcA→GlcNS ±6S
6 sequence to GlcA→GlcNS 3S ±6S, thereby converting HS^{act} precursor to HS^{act}.

1 28. A method as in any one of claims 19-28 wherein said sulfate donor is PAPS.

1 ~~29.~~ A non-human animal model, wherein a genome of said animal, or an ancestor
2 thereof, wherein said recombinant construct has introduced a modification into said
3 genome, said modification selected from the group consisting of insertion of a nucleic
4 acid encoding at least a functional fragment of a conspecific wild type 3-OST protein,
5 insertion of a nucleic acid encoding at least a functional fragment of a transpecific
6 allelic variant of the 3-OST sequences, insertion of nucleic acid encoding at least a
7 functional fragment of an allelic variant of 3-OST sequence, inactivation of an
8 endogenous 3-OST gene, and insertion by homologous recombination of a reporter
9 gene coupled to 3-OST transcriptional elements.

1 30. An animal as in claim 29 wherein said modification is insertion of nucleic acid
2 encoding at least a functional fragment of wild type 3-OST selecting from the
3 sequence consisting of the SPLAG-domain, the cysteine-binding peptide loop, and the
4 ~260 residue ST domain.

1 31. An animal as in claim 29 wherein said animal is selected from the group
2 consisting of rats, mice, hamsters, guinea pigs, rabbit, dogs, cats, goats, sheep, pigs,
3 and non-human primates.

1 32. An animal as in claim 29 wherein said animal is an invertebrate.

1 ~~33.~~ A method of producing antibodies which selectively bind to a 3-OST protein
2 comprising the steps of

3 administering an immunogenically effective amount of a 3-OST epitope to an
4 animal;
5 allowing said animal to produce antibodies to said epitope; and
6 obtaining said antibodies from said animal or from a cell culture derived
7 therefrom.

1 ~~34~~ A substantially pure preparation of antibody which selectively binds to an
2 epitope of a 3-OST protein.

1 35. A substantially pure preparation of an antibody as claimed in 34 wherein said
2 antibody selectively binds to at least a fragment of 3-OST.

1 36. A cell line producing an antibody of any one of the claims 34.

1 ~~37~~ A method for identifying compounds which can modulate the expression of a
2 ~~3-OST~~ gene comprising steps of
3 providing a cell expressing a nucleic acid under the control of a 3-OST
4 regulatory sequence;

5 contacting said cell with at least one candidate compound; and
6 assaying for a change in the in the expression of said nucleic acid.

1 38. The method of claim 37, wherein said nucleic acid comprises a marker gene
2 and a 3-OST gene

1 39. The method of claim 37, wherein said said assaying step comprises detecting a
2 change in 3-OST mRNA level.

1 40. The method of claim 37, wherein said said assaying step comprises detecting a
2 change in 3-OST protein encoded by said nucleic acid.

1 ~~41~~ A method of determining partial sequence information for complex
2 polysaccharides comprising the steps of:
3 contacting a first sample of polysaccharide with at least one ligand which
4 binds polysaccharides in a sequence specific manner;
5 contacting the resulting polysaccharide-ligand complex with at least one agent
6 that modifies complex polysaccharides;
7 contacting a second sample of polysaccharide with the same modifying agent;

8 comparing said first and second samples for ligand-specific inhibition of
9 modifications caused by said modifying agent.

1 42. The method of claim 41, wherein said complex polysaccharide is a
2 glycosaminoglycan.

1 43. The method of claim 41, wherein said ligand is catalytically inactive.

1 44. The method of claim 41, wherein said ligand is an inactive 3-OST.

1 45. The method of claim 41, wherein said agent that modifies polysaccharides is
2 selected from the group consisting of epimerases, lyases, sulfotransferases, N-
3 acetyltransferases, N-deacetylases, epimerases.

1 46. The method of claim 45, wherein said modifying agent is a sequence specific
2 degrading agent.

1 47. The method of claim 45, wherein said modifying agent is a non-sequence
2 specific degrading agent.

1 48. The method of claims 46, wherein said degrading agent is a lyase.

1 49. The method of claim 47, wherein said non-sequence specific degrading agent
2 nitrous acid.

1 50. The method of claim 45, further comprising affinity purifying said modified
2 first and second samples.

1 51. The method of claim 45, wherein the step of comparing includes a comparison
2 of size profiles.

1 ~~52.~~ A method of determining partial sequence information for complex
2 polysaccharides comprising the steps of:
3 contacting a first sample of complex polysaccharides with a 3-OST protein in
4 the presence of a sulfate donor under conditions which permit sulfation by said 3-
5 OST;
6 contacting said first sample and a second sample with at least one enzyme
7 which cleaves polysaccharides in a sequence-specific manner;
8 determining the size profiles of the resulting fragments.

1 53. The method of claim 52, wherein the determining the size profile step further
2 comprises the step of comparing said first sample to a second sample cleaved by the
3 same enzymes.

1 54. The method of claim 52, wherein said enzymes which degrade polysaccharides
2 in a sequence specific manner are selected from the group consisting of
3 polysaccharide lyases, heparinase I, heparinase II, and heparinase III

1 55. A method of determining partial sequence information for a sample containing
2 complex polysaccharides comprising the steps of:
3 contacting said sample of polysaccharide with a 3-OST protein which lacks
4 enzymatic function with a under conditions which permit said 3-OST protein to bind
5 to said polysaccharide in a sequence specific manner;
6 applying said sample to an affinity column;
7 applying degrading agents to said column;
8 analyzing the resulting degradation products.

1 56. The method of claim 55, further comprising repeating the steps applying
2 degrading agents and analyzing using a series of different sequence specific
3 polysaccharide cleavage enzymes.

1 57. An isolated nucleic acid comprising a genetic regulatory sequences of a 3-OST
2 operably joined to a marker gene.

1 58. A host cell transformed with the isolated nucleic acid of claim 57, or a
2 descendent thereof.

1 59. A method of identifying compounds capable of modulating the expression of a
2 3-OST comprising contacting candidate compound seith the transformed host cell of
3 claim 58 and assaying for changes in expression of said marker.

1 60. A method as in claim 59, wherein said regulatory sequences comprise the 5'
2 untranslated region of SEQ. ID NO: 16.